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Lake Trout Early Life Stage Mortality: Interactions of the Nutrient Thiamine and Dioxin-Like PCBs and Their Mixtures Found in Green Bay

Final Report of Interagency Agreement 14-48-0009-98-978
31 October 1999

Prepared For U.S. Fish and Wildlife Service
Green Bay Field Office, Ecological Services
1015 Challenger Ct., Green Bay, WI 54311-8331

Contact: Donald Tillitt, PhD., Principal Investigator

U.S. Department of the Interior
U.S. Geological Survey

NOTE TO READERS

These reports were prepared under interagency agreements between the Columbia Environmental Research Center, USGS, and the Green Bay Ecological Services Field Office, USFWS. Original funding was supplied by the Division of Environmental Contaminants, USFWS. In addition to having applications throughout the Great Lakes Ecosystem, a specific objective of these studies was to develop information related to the Green Bay/Fox River NRDA. Interpretation of these results within the context of this NRDA can be found on the official website related to this case at:

http://www.fws.gov/r3pao/nrda/

Specifically the document discussing these results is the Fish Injury Report

R Kravitz
r. Stromborg

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1.0 Executive Summary

The purpose of these studies was to determine the potential for interactions between thiamine deficiency and polychlorinated biphenyl (PCB)-induced toxicity on the development of lake trout embryos and fry. The potential interaction between thiamine deficiency and PCB-induced toxicity is important to the Green Bay/Fox River NRDA because, if it is true, lake trout in Lake Michigan may be more susceptible than hatchery-reared stocks. As a result, the injury to lake trout populations from the PCBs and other dioxin-like compounds released into the Fox River and subsequently into Green Bay, may have been greater than expected from laboratory studies.

The experiments reported here were an extension of the studies conducted in 1996-1997. Technical difficulties during the course of those studies made interpretation of the results for the injury determination impossible. So, the studies in the current report were again designed to test the hypothesis of an interaction between low thiamine content and elevated PCB content in lake trout fry mortality. Eggs from lake trout containing high or low thiamine were injected with graded doses of a dioxin-like PCBs (PCB 126), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), or a PCB-containing extract of walleye from the Fox River. Thiamine and the contaminant concentrations were measured for the treatments. The survival and development of the lake trout were monitored through swim-up. The symptoms of PCB-induced toxicity in the sac fry (hemorrhage, yolk-sac edema, and craniofacial anomalies) were monitored between hatch and swim-up stages of the lake trout.

Dose related increases in fry mortality were observed with PCB 126 (3,3',4,4',5-pentachlorobiphenyl) and the median toxicity values obtained in our studies (20 and 27 ng/g egg) confirm the one study from the literature. The complex mixture of organic chemicals extracted from the Fox River walleye caused dioxin-like toxicity in early life stages of lake trout. The greatest dose tested (157 pg TEQs/g) caused deformities in all of the fry and nearly complete mortality. The next lower dose of the walleye extract (15 pg TEQs/g) caused increases in deformities and mortality in some groups, but the increases were not statistically significant. This dose appears to be just below the threshold for dioxin-like toxicity for lake trout fry survival, which is again consistent with the literature. The result of the injection studies with the walleye extract are also consistent with an additive model of toxicity, and support the continued use of the TEF/TEQ approach for assessment of dioxin-like effects in developing lake trout.

These studies confirmed that the lake trout is one of the most sensitive species toward the adverse affects of dioxin-like chemicals, including PCBs. However, these studies failed to support our original hypothesis that low thiamine status in lake trout eggs would further enhance the sensitivity of this species toward dioxin-like toxicity. The data did not support the contention that low thiamine content in lake trout embryos and fry might exacerbate the effects of PCBs or dioxins on fry survival. Various technical factors compromised the studies and a rigorous test of the hypothesis was not possible.

2.0 Introduction

Polychlorinated biphenyl (PCB) contamination in the lower Fox River and Green Bay, Wisconsin has lead to the concern that lake trout *(Salvelinus namaycush)* populations may have been adversely affected by this contamination. Lake trout are exposed to PCB contamination originating from historic discharges of paper mills on the Fox River (Hagler Bailly Consulting 1996). The linkage between reproductive injuries suffered by top predators in aquatic food webs and the contaminated sediments at the base of those food webs has been demonstrated by over three decades of research (Allen; Sullivan; Persson, and other members of the Technical Advisory Commitee 1987;Bierman; DePinto; Young; Rodgers; Martin; Raghunathan, and Heinz 1992;Marti and Armstrong 1990;Thomann and Connolly 1984;USEPA 1993;Willford; Bergstedt; Berlin; Foster; Hesselberg; Mac; Passino; Reinert, and Rottiers 1981). The importance of the effects of contaminated sediments for natural resources management was recognized by the Great Lakes Fish Health Committee (Great Lakes Fish Health Committee 1994).

Lake trout embryos are known to be sensitive to the lethal effects of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and planar PCBs (pPCBs), collectively referred to as planar halogenated hydrocarbons (PHHs). Although many factors impinge on the stability of such a fish population, historic concentrations of contaminants in the eggs of lake trout have been great enough to cause overt toxicity, independent of other factors (Raloff, 1997). Recently, it has been hypothesized that thiamine deficiency has caused swim-up syndrome in some populations of Atlantic salmon from the Finger Lakes region of New York and in lake trout from the Great Lakes (Fitzsimons et al. 1995). The symptomatology of the swim-up syndrome associated with thiamine deficiency and the signs of PHH-induced toxicity in salmonids share a number of similarities (Fisher et al. 1995; Walker et al. 1994). Both of these stressors may be acting through a similar mechanism and as such may be exacerbating the effect of each of these factors on their own. Therefore, it is the purpose of these studies to determine the potential interactions of thiamine deficiency and PHH toxicity on the development of lake trout embryos. This evaluation of the role of PCB contamination in lake trout reproductive failure will guide Service efforts in lake trout restoration in the Great Lakes.

3.0 Experimental Design

The specific objectives of these studies were addressed through the use of egg injection techniques through which Lake trout eggs were injected with nanoliter volumes of contaminants and the developing embryos were monitored through swim-up. The sources of lake trout eggs were: (1) feral trout netted in and around Sturgeon Bay, Lake Michigan and (2) the National Research and Development Lab, Leetown Science Center, Wellsboro, PA. The Wellsboro female lake trout were maintained on diets formulated with constituents low in thiamine and containing bacterial thiaminase. These diets were utilized to induce a hypovitaminosis B_1 in the female lake trout that would result in egg thiamine deficiencies approximating the low thiamine levels found in eggs from the feral lake trout population(Honeyfield et al.1998). Eggs from one Lake Michigan female and four Wellsboro females were included in the injection study. The chemicals and chemical mixtures (N=3) included: (1) 2,3,7,8 TCDD, (2) PCB126, and (3) a Green Bay/Fox River Walleye Extract. Variations of thiamine (B_1) supplementation (N=3) were (a) no supplementation, (b) supplementation with 75mg/L or (c) with 750mg/L thiamine at water hardening. The treatments in the experimental design matrix are described in Table 1, below.

Table 1. Experimental Design Matrix of Treatment Combinations for Studies into the Interactive Effects of Thiamine and Dioxin-like Chemicals on Lake Trout Early-Life Stage Mortality

Source of Eggs *B_1 supplementation*	Chemical Exposure		
	TCDD	PCB 126	Walleye Extract
Lake Michigan (eggs from 1 female) *750 mg/L B_1*			N=1
Wellsboro, PA **(eggs from 4 females)**			
no B_1	N=1		
75 mg/L B_1	N=1	N=1	N=1
750 mg/L B_1	N=1	N=1	N=1

N = the number of complete dose-response curves for each treatment combination

Complete dose-response curves were developed for each of the treatment combinations in the matrix above. The curve for each chemical consisted of five graded doses, a dose of the vehicle with no chemical and an uninjected control. The individual injection doses for the chemicals used were:

2,3,7,8 TCDD - Control, 0, 7, 12, 24, 48, 122 pg. TCDD/gram (wet weight) of egg,

PCB 126 – Control, 0, 3.5, 7, 14, 28, 56 ng PCB/gram (wet weight) of egg , and

Walleye Extract – Control, 0, 0.0045, 0.044, 0.44, 4.4 and 45 eggEQ/egg.

The chemical solutions of each compound in the study were formulated to a concentration such that, when suspended in the triolein vehicle, each of the desired doses could be achieved at an injection volume of 20 nl. A single dose level, Walleye Extract (45 eggEQ/egg), was administered as 40 nl injections volumes due to the low solubility of the extract solution in the injection vehicle.

3.0 Methods

3. Chemicals

Triolein

Triolein (95%) purity, Sigma Chemical Company) is the lipid vehicle for all injection solutions. Prior to it's inclusion in the injection solutions the triolein was filtered to sterility through disposable sterile syringe filters (25 mm, 0.20 microns, and cellulose acetate membranes) and stored in sterile tubes until use.

Methylene chloride

Methylene chloride (Fisher Scientific) was used in a 1:1 ratio with the triolein to prepare the contaminant injection solutions. Methylene chloride serves to disperse the contaminant more evenly in the triolein vehicle.

2,3,7,8 TCDD

The 2,3,7,8 TCDD source solution was obtained from previously used CERC stocks[1]. TCDD solutions were prepared as previously described in the S.O.P. (Tillitt; Anderson, and Holey 1996) with the following exceptions: The injection solutions were prepared from serial dilutions of the

1 MSC stock solution 166W, CERC, Columbia, MO

study chemical dispersed in 1:1 Methylene Chloride / Triolein solution. The resultant volumes were then reduced to their final concentration by evaporation of all the methylene chloride under nitrogen, as per the description in the S.O.P. for egg injection of lake trout. The calculations of the doses and serial dilutions for the TCDD solutions used in these studies are shown below (Table 2).

Table 2. Calculations for the preparation of the 2,3,7,8 TCDD injection solutions.

Solution Label	Injection Dose pg/g $_{egg}$ *(nominal)*	Concentration pg/µl *(nominal)*	Measured Concentration pg/µl	Measured Dose pg/g $_{egg}$
1	160	720	550	122
2	80	360	215	48
3	40	180	110	24
4	20	90	55	12
5	10	45	30	7
6	0	0	0	0

These TCDD calculations are based on using a 114 µl volume of the 166W stock solution (7870 pg/µl *nominal concentration*) to formulate the most concentrated solution (1) with progressive serial dilution to all of the lower concentrations. The dosages are based on an injection volume of 20nl into lake trout eggs with an average mass of 0.09 grams. The measured concentrations are based on high resolution, gas chromatography-mass spectrometry analysis of the dosing solutions. The measured dose given to the eggs was based on the measured concentrations in the dosing solutions.

PCB 126

The PCB 126 (3,3',4,4', 5-pentachlorobiphenyl) stock solution was obtained from AccuStandard[1]. The PCB solutions were prepared as previously described (Tillitt 1999; Tillitt and others 1996). The injection solutions were prepared from serial dilutions of the study chemical dispersed in 1:1 Methylene Chloride / Triolein solution. The resultant volumes were then reduced to their final concentration by evaporation under nitrogen. The tabular representation of the PCB calculations and serial dilutions for the injection solutions are found below (Table 3).

1 C-126S-TP, AccuStandard Inc.

Table 3. Concentrations and doses of the PCB 126 injection solutions.

Solution Label	Concentration pg/µl *(nominal)*	Injection Dose pg/g $_{egg}$ *(nominal)*
1	252	56
2	126	28
3	630	14
4	32	7
5	16	3.5
6	0	0

These PCB calculations are based on using a 2520 µl volume of the stock solution (100 ng/µl *nominal concentration*) to formulate the most concentrated solution (1) with progressive serial dilution to all of the lower concentrations. The dosages are based on an injection volume of 20nl into lake trout eggs with an average mass of 0.09 grams. Results of the measurements of PCB 126 in the dosing solutions were not available at the time of this report.

Walleye Extract

Walleye extracts were prepared as previously described (Tillitt 1999). The selected doses were chosen to approximate the natural exposure of the eggs to the chemical mixtures present in fish from the Fox River, Green Bay, Wisconsin at the time of the walleye collection in 1996.

Dose of the Walleye Extract Injected

The concentrations of the walleye extract injected into the lake trout eggs can be expressed as gram-equivalents (g-EQs), egg-equivalents (egg-EQs), or 2,3,7,8-tetrachlorodibenzo-p-dioxin-equivalents (TEQs) (Table 4). The doses were normalized to the mass of the egg on a wet weight basis or to the lipid content of a lake trout egg. The most useful doses for determining dose response relationships were egg-EQ/egg and pg-TEQ/g egg. The measure of egg-EQ/egg is a simple multiplier in which 1 egg-EQ is the amount of contaminants expected to be in 1 lake trout egg. Thus, 2 egg-EQ/egg is two times the amount expected in a lake trout egg at the time at which the walleye were sampled, and so on.

Table 4. Dose calculations: Fox River Walleye Extract Injected into Lake Trout Eggs.

		Dosing Solution				
		1	2	3	4	5
Volume Injected	(uL/egg)	0.04	0.02	0.02	0.02	0.02
Extract conc.	(g-EQ/uL)	36	7.1	0.71	0.071	0.0072
Dose	(g-EQ/egg)[1]	1.44	0.142	0.014	0.0014	0.00014
	(g-EQ/g egg)[2]	16.0	1.58	0.16	0.02	0.002
	(egg-EQ/g egg)[3]	500	49	4.9	0.49	0.05
	(egg-EQ/egg)[4]	45	4.4	0.44	0.044	0.0045
	(pg TEQ/g egg)[5]	157	15	1.5	0.15	0.016

Calculations of the doses injected into the lake trout eggs were based on the volume injected into the eggs, the extract concentrations (Table 3), an average measured egg mass of 0.09 g wet wt./egg, the lipid content of the walleye extract (14% or 0.14 g-lipid/g), and the lipid content of lake trout eggs (5% or 0.05 g-lipid/g). 1) g-EQ/egg = (g-EQ of extract/uL)(uL/egg); 2) g-EQ/g egg = (g-EQ/egg)/(g egg/egg); 3) egg-EQ/g egg = (g-EQ/g egg)(0.14 g-lipid/g-EQ extract wet wt.)/(0.09 g egg wet wt./egg)(0.05 g-lipid/g egg); 4) egg-EQ/egg = (egg-EQ/g egg)(0.09 g wet wt./egg); 5) pg TEQ/g egg = (g-EQ/g egg)(9.8 pg TEQ/g-EQ of extract).

The measure of dose as pg TEQ/g egg was also used to characterize dose response relationships. The TEQ measure of dose is based on the TEQ/TEF approach and assumes that the dioxin-like chemicals present in the extract will act in an additive fashion (van den Berg et al. 1998). The TEQ approach of measuring dose allows comparison with other studies which have been conducted in lake trout and rainbow trout with PHH-induced early life stage toxicity (Van den Berg and others 1998; Spitsbergen et al. 1991; Walker et al.1996; Walker et al.1994; Zabel et al. 1995). The doses which were selected for this study were chosen to bracket the amount of contaminants in lake trout from approximately 0.5% to 45 times the amount expected in a lake trout egg at the time of the sampling of the walleye (Table 4). The doses were also selected to bracket the concentrations of dioxin-like potency known to effect embryo survival and development from previous studies with lake trout (Walker et al. 1996; Elonen et al. 1998). The LD50 of TCDD for lake trout early life stage survival has been measured to be 80 pg/g (Walker et al. 1994; Wright and Tillitt, unpublished data). The concentrations of TEQs in the doses of the walleye extract doses given to the lake trout eggs were 0.016 - 126 pg TEQ/g egg (Table 4).

3.2 Injection Needles

In previous years, studies at our lab utilized needles fabricated from aluminosilicate glass capillary tubes with an outer diameter (O.D.) of 1.00mm and an inner diameter (I.D.) of 0.53mm. When prepared properly, injection needles made from these tubes have marginally sufficient strength and toughness for successful injections. Capillary tubes of these dimensions became unavailable from our original domestic supplier[1] and tubes with thinner walls (1.00mm O.D., 0.68mm I.D.) from this supplier possessed inadequate strength for dependable use in our studies. A European supplier[2] was located for glass with the original dimensions (1.00mm O.D., 0.5mm I.D.) tubes but these capillary tubes demonstrated a high degree of variability in quality control and "batch consistency". The resulting strength and toughness of the needles from the European source capillaries were found to be inadequate for injection studies also. The inability to acquire high quality capillary tubes and the resulting high rate of needle breakage during injection reduced the number of injected eggs that could be included in this study.

3.3 Incubator Environment

Water Temperature

Incubator water temperature was held at 8 ^{0}C (± 0.5^{0}) with a Neslab® external water chiller system[3]. Incubator temperature was stabilized via an automated flow diversion system, which protected the eggs from any variations in water temperature. Temperature probes were also linked to an alarm system for continuous monitoring and around-the-clock notification of lab personnel concerning potential temperature fluctuations in the supply water. A constant temperature work area for egg fertilization and handling was also fabricated for this study to minimize the disruption of the incubator environment and maintain a uniform and stable environment during all egg handling phases of the study. Computerized monitoring of water parameters was instituted for this study[4]. Values of temperature (7.53 ± 0.54 ^{0}C), pH (7.68 ± 0.06), conductivity (0.532 ± 0.129 mS/cm), salinity (0.258 ± 0.080 ppt) and dissolved oxygen (8.91 ± 0.639 mg/l) were recorded hourly throughout the course of the study.

Water Flow

All incubators were held at an average flow of 4 L/min throughout the duration of the study. Incubator flow was optimized through modification of the incubator trays. A 5 cm extension was

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1 AF100-58-10, Sutter Instruments, Novato, CA
2 SM100F-10, Clark Medical Instruments, Pangbourne Reading, England
3 HX-900 Water-Cooled Flow-Thru Chiller, Neslab Instruments, Newington, NH
4 Datasonde

added to all trays to maximize the efficiency of the cascading flow system at all flow rates. The egg baskets were redesigned from previous years to optimized flow to the eggs. The egg baskets used in this year's study were cylindrical in shape, 5 cm in diameter, 5 cm in height, with 2 mm square mesh on the sides and bottom. Antifungal procedures for this study included thorough cleaning and sterilization of incubators and trays before introduction of the eggs, diligent removal of dead eggs and debris, and intermittent formalin treatments. The treatments consisted of adding formalin (37% formaldehyde)[1] to the water flow via a drip system at a rate of 4.5 mls/min for 15 minutes. This brought the formalin concentration in the incubator flow to 1667 ppm. This treatment was administered 3 times per week and discontinued at eye up.

3.4 Fertilization of the lake trout eggs

Shipping mortality on the fertilized eggs shipped from Wellsboro and Lake Michigan was commonly 30-75% in all the egg batches and seriously reduced the number of viable eggs below that needed for dose response injection studies. Therefore, we altered our protocols to include the shipment of unfertilized eggs and milt to CERC. Eggs received in November (received on 11/6 and 11/13) were fertilized after arrival at CERC.

Upon arrival at CERC, the unfertilized eggs were brought slowly (<1°C/hr) from shipping (arrival) temperature (5-8°C) to fertilization temperature (10°C). Eggs were place in dry glass containers and mixed with pooled milt samples from 2 males. The eggs were then stirred gently for 30 seconds and allowed to stand undisturbed for two minutes. The eggs were then rinsed and divided into two equal portions. The two aliquots were then water hardened in thiamine solutions of either 75 or 750 mg/L for two hours in the dark. The solutions were gently aerated for the duration of the treatment. The batches were then rinsed and placed in an aerated solution of 100ppm Iodine for 10 minutes. They were slowly brought to (8° C) and placed in the incubators.

All egg batches used in the study were fertilized on the day of arrival except for a single batch (T5-CBT-F11-11/18) which was slowly cooled to 5° C for storage from 11/13 to 11/18. This batch was then fertilized on 11/18 with fresh milt (collected from Wellsboro on 11/16) using the protocol described above.

4.0 Results and Discussion

Several shipments of lake trout eggs were received in the fall of 1998 as potential candidates for the injection study component of the Fox River/Green Bay NRDA 1998-1999 lake trout studies.

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1 Sigma Chemical

We received eggs on 10/22/98 from Wellsboro, eggs from 11 females: 10/21-F-1-BT through 10/21-F-11-BT that received a low thiamine diet supplemented with bacterial thiaminase (BT) (Honeyfield et al. 1998). The eggs were fertilized immediately after collection at the hatchery prior to shipment and each egg batch was divided into two groups; with and without thiamine treatment (750mg/L at water hardening). Egg batches were also received that same day from Wellsboro, eggs from six females, 10/21-F-8-CBT through 10/21-F-13-CBT that were fed on the casein/gelatin diet (Dale Honeyfield, personal communication). All of these eggs were fertilized immediately after collection at the hatchery prior to shipment to the CERC, with no thiamine treatment.

On 10/28/98, 10/29/98, and 10/30/98 we received daily shipment of egg batches from the feral Lake Michigan lake trout collect and fertilized immediately upon collection on 10/27 (F1-F9), 10/28(F10-F26), and 10/29 (F27-F32), respectively. All the egg batches from Lake Michigan were divided into two groups per female, were water hardened either with or without thiamine treatment (750mg/L at water hardening), and shipped to the CERC.

Shipping mortality on the fertilized eggs shipped from Wellsboro and Lake Michigan was commonly 30-75% in all the egg batches and seriously reduced the number of viable eggs required for the dose response injection studies. Therefore, subsequent batches of eggs were shipped unfertilized, as eggs and milt. Eggs received in November were fertilized after arrival at CERC.

On 11/06/98 we received egg batches from 7 Wellsboro females, T6- BT-F-6 though T6-BT-F-12 that received a low thiamine diet supplemented with bacterial thiaminase (BT). These eggs were shipped unfertilized and were fertilized post-shipment at CERC.

On 11/13/98 we received egg batches from 8 Wellsboro females, T5- CBT-F-3 though T5-CBT-F-12 that received a purified casein/gelatin diet supplemented with bacterial thiaminase (CBT). These eggs were shipped unfertilized and were fertilized at CERC.

The fertilization technique used at CERC on the 11/06, and 11/13 shipments were patterned after the SOP for Lake Trout (1996) with minimal modifications. See the outline of the procedures given above in the Methods Section.

From these shipments, eggs were selected for injections based on shipping mortality, thiamine content, needle availability, and age post-fertilization. The actual injection studies conducted with the various lake trout eggs are given below (Table 5).

Table 5. The fertilization rates, control mortality, thiamine content and treatments of lake trout eggs used in the Fox River/Green Bay NRDA 1998-1999 studies.

Egg Source	Batch/ Female	Contaminant injected	Fertilization Rate [a] (%)	Control Mortality [b] (%)	Thiamine Treatment [c] (mg/L)	Thiamine Content (nmol/g) Unfertilized	Mid-Study
Wellsboro Day 0 = 10/21/98	10/21-F-11-BT-0-1	2,3,7,8 TCDD	58.3	81.8	0	0.745	.77[1]
Wellsboro Day 0 = 10/21/98	10/21-F-11-BT-1-1	2,3,7,8 TCDD	50.7	18.75	750	0.745	5.201[1]
Lake Mich. Day 0 = 10/28/98	10/28-F-10-F-T-1-1	Walleye Extract	37.7	0	750	2.070	10.255[2]
Wellsboro Day 0 = 11/18/98	T5-CBT-F11	PCB 126	49.1	4	*75	0.963	5.584[3]
Wellsboro Day 0 = 11/18/98	T5-CBT-F11	PCB 126	42	16	750	0.963	6.047[3]
Wellsboro Day 0 = 11/6/98	T6-BT-F7	Walleye Extract	53.3	0	75	.205	.657[4]
Wellsboro Day 0 = 11/6/98	T6-BT-F7	Walleye Extract	64.2	3.8	750	1.205	7.539[4]
Wellsboro Day 0 = 11/13/98	T5-CBT-F12	2,3,7,8 TCDD	36.7	31	*75	1.285	3.967[5]

[a] The % fertility in uninjected Control group.
[b] The % mortality in uninjected Control group at the end of the study (155 days).
[c] Thiamine treatments at the stated concentration at water hardening (2 hour exposure).
* Upon analysis, these solutions were found to contain 750mg/L thiamine.
[1] Measured thiamine concentration 43 days post treatment.
[2] Measured thiamine concentration 37 days post treatment.
[3] Measured thiamine concentration 15 days post treatment.
[4] Measured thiamine concentration 28 days post treatment.
[5] Measured thiamine concentration 20 days post treatment.

Dose-Response Results

PCB 126

The eggs from one Wellsboro female (T5 CBT F11) were utilized for a PCB 126 dose-response study. The eggs from this female had a pre-fertilization thiamine concentration of 0.96 nmol/g.

Figure 1. Total Cumulative Survival (%) of Lake Trout Eggs and Fry from Wellsboro, Treated with 750 mg/L Thiamine (nominal) and Exposed to PCB 126. Female/Egg Batch: T5-CBT-F-11.

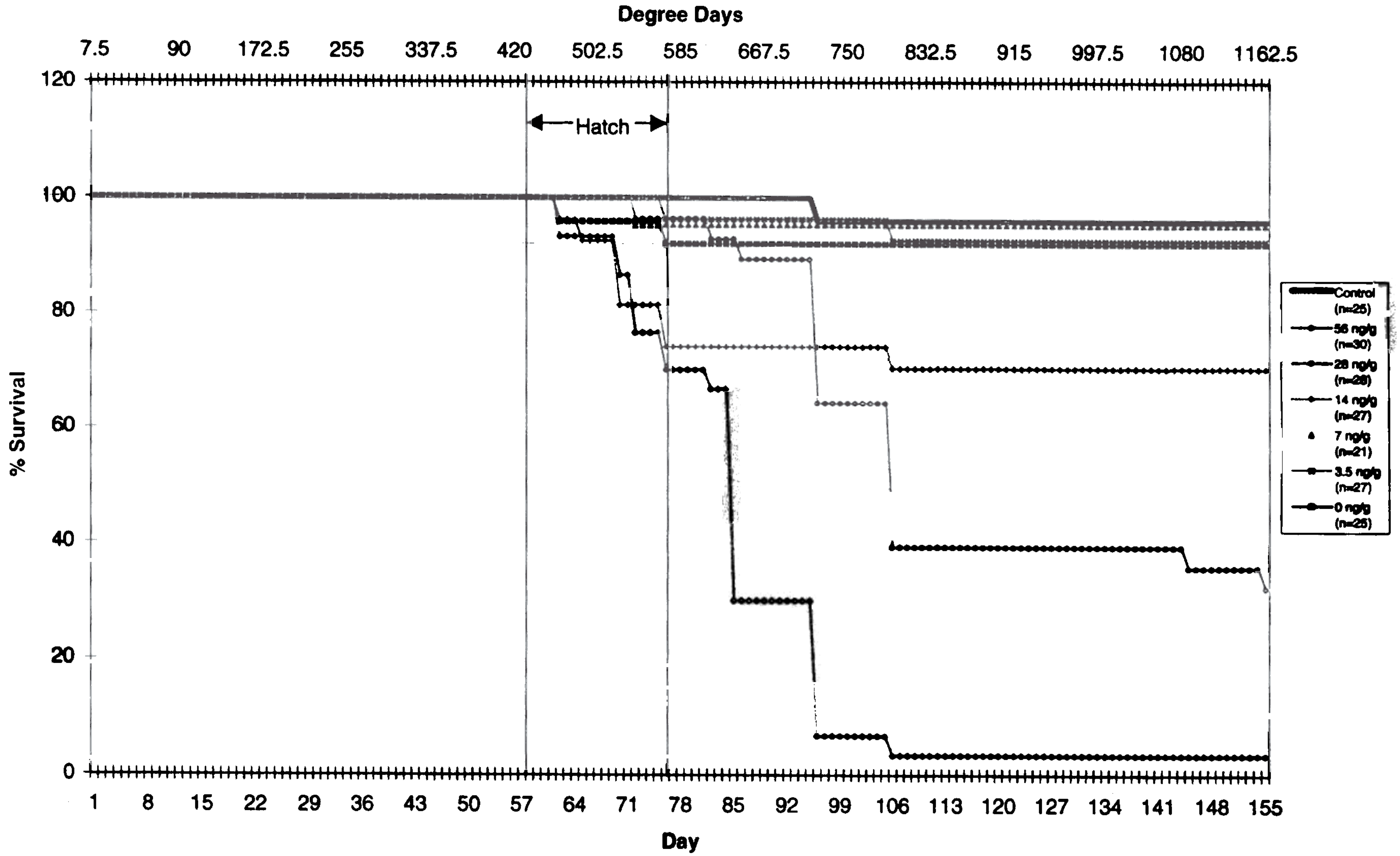


Figure 2. Total Cumulative Number of Survivors of Lake Trout Eggs and Fry from Wellsboro, Treated with 750 mg/L Thiamine (nominal) and Exposed to PCB 126.
Female/Egg Batch: T5-CBT-F-11.

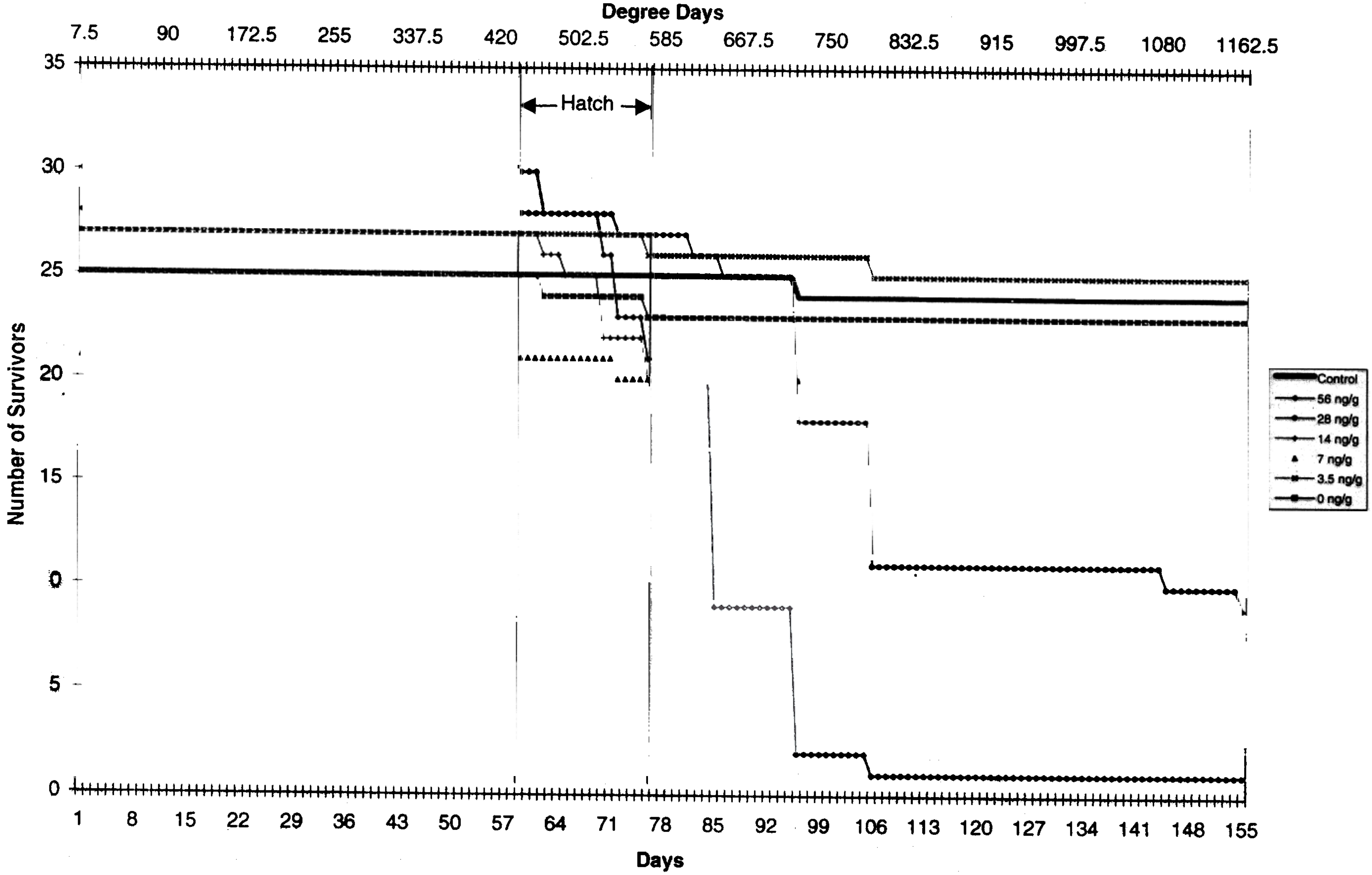


Figure 3. Total Cumulative Survival (%) of Lake Trout Eggs and Fry From Wellsboro, Treated with 750 mg/L Thiamine and Exposed to PCB 126. Female/Egg Batch: T5-CBT-F-11.

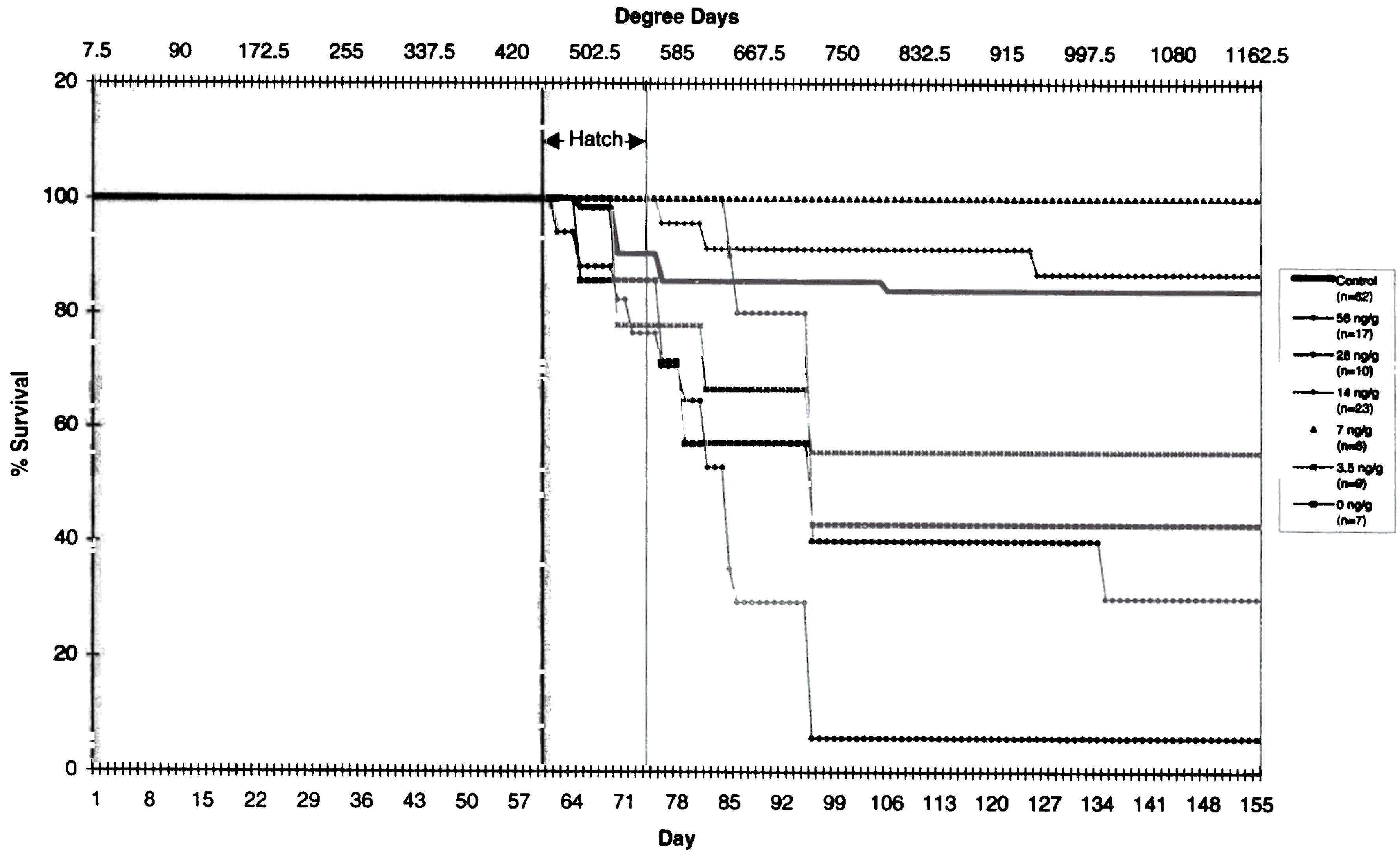
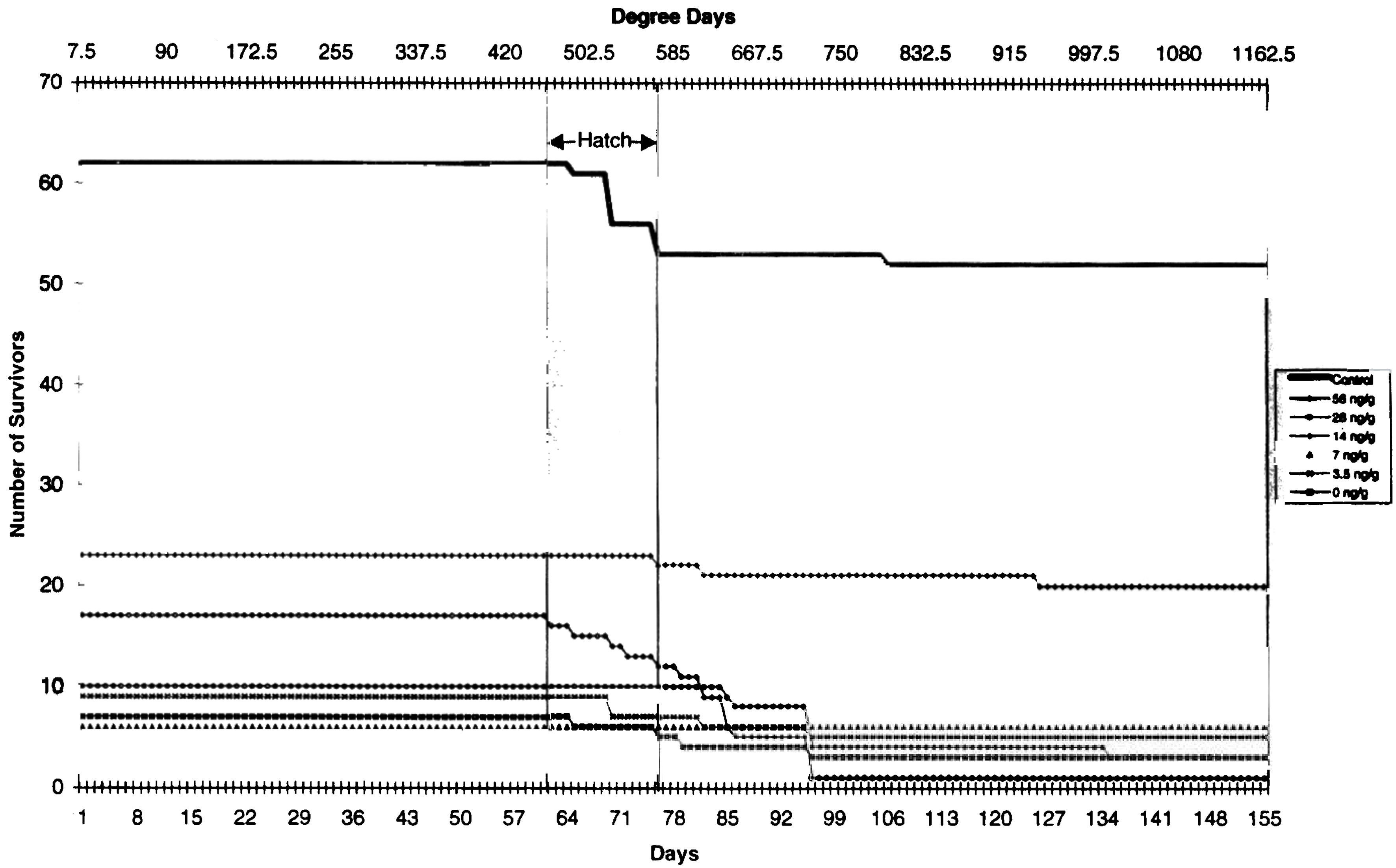


Figure 4. Total Cumulative Number of Survivors of Lake Trout Eggs and Fry From Wellsboro, Treated with 750 mg/L Thiamine and Exposed to PCB 126. Female/Egg Batch: T5-CBT-F-11.



The LD_{50} values estimated in lake trout injected with PCB 126 were consistent with the existing literature on this congener. Zabel et al. (1995) reported an LD_{50} value of 29 ng/g egg for PCB 126 in lake trout. We estimated a nearly identical median lethal value (Table 6) in the lake trout treated with 750 mg thiamine/L (nominal). The lake trout which received the lower nominal thiamine treatment during water hardening and contained slightly less thiamine in their eggs at 15 day post fertilization (Table 5) had a lower LD_{50} value of 20 ng/g egg (approximately one third less). The two LD_{50} values estimated for PCB 126 in lake trout fry over the course of this study, were not statistically different from one another.

2,3,7,8-TCDD

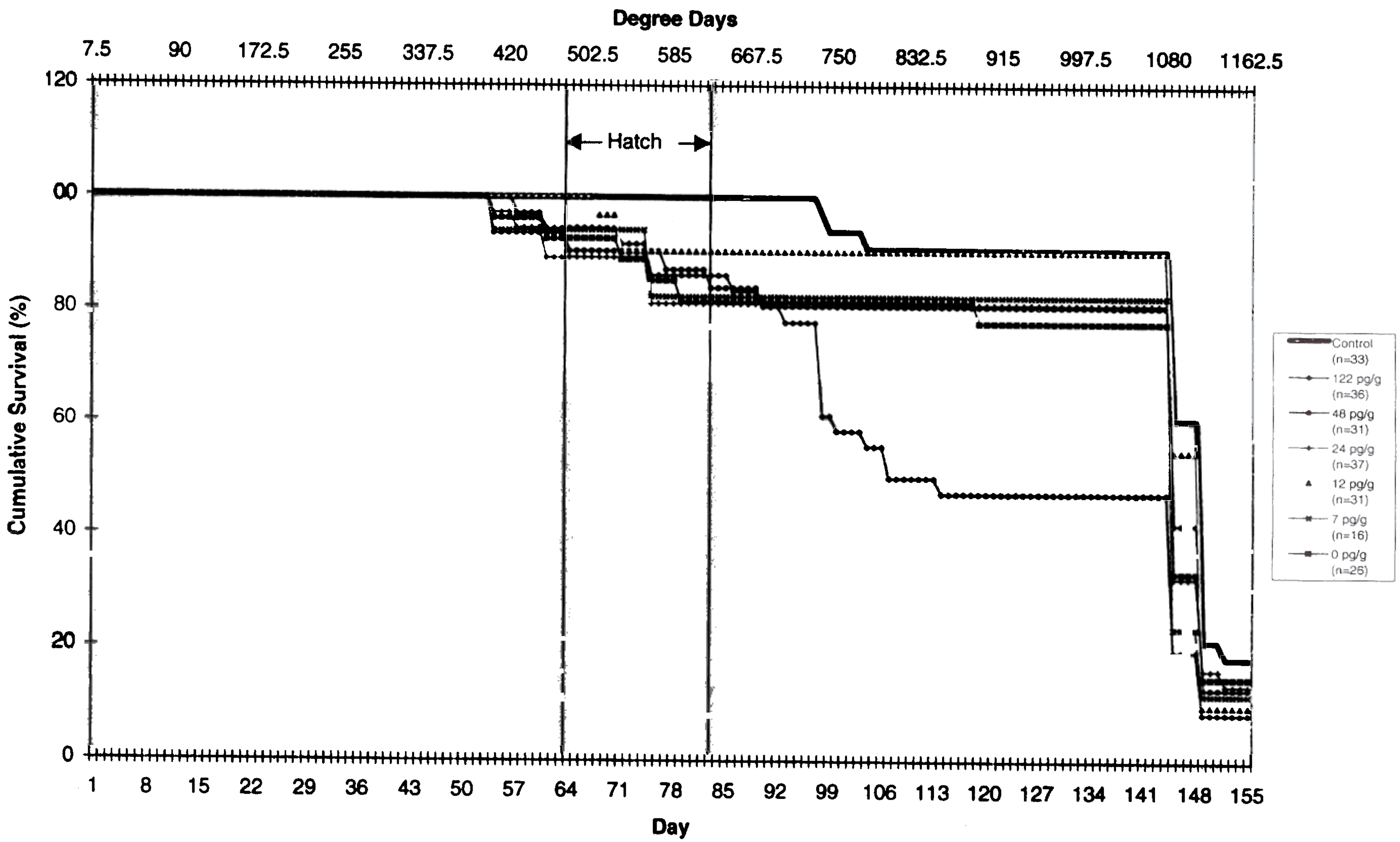
Eggs from two Wellsboro females (10/21-F11-BT and T5-CBT-F12) were dosed with TCDD. The eggs from female 10/21-F11-BT had a pre-fertilization thiamine concentration of 0.75 nmol/g and the eggs from T5-CBT-F12 had a pre-fertilization thiamine level of 1.29 nmol/g. The eggs labeled 10/21-F11-BT were fertilized in Wellsboro and experienced a great deal of mortality, presumably due to the stresses associated with the shipping process.

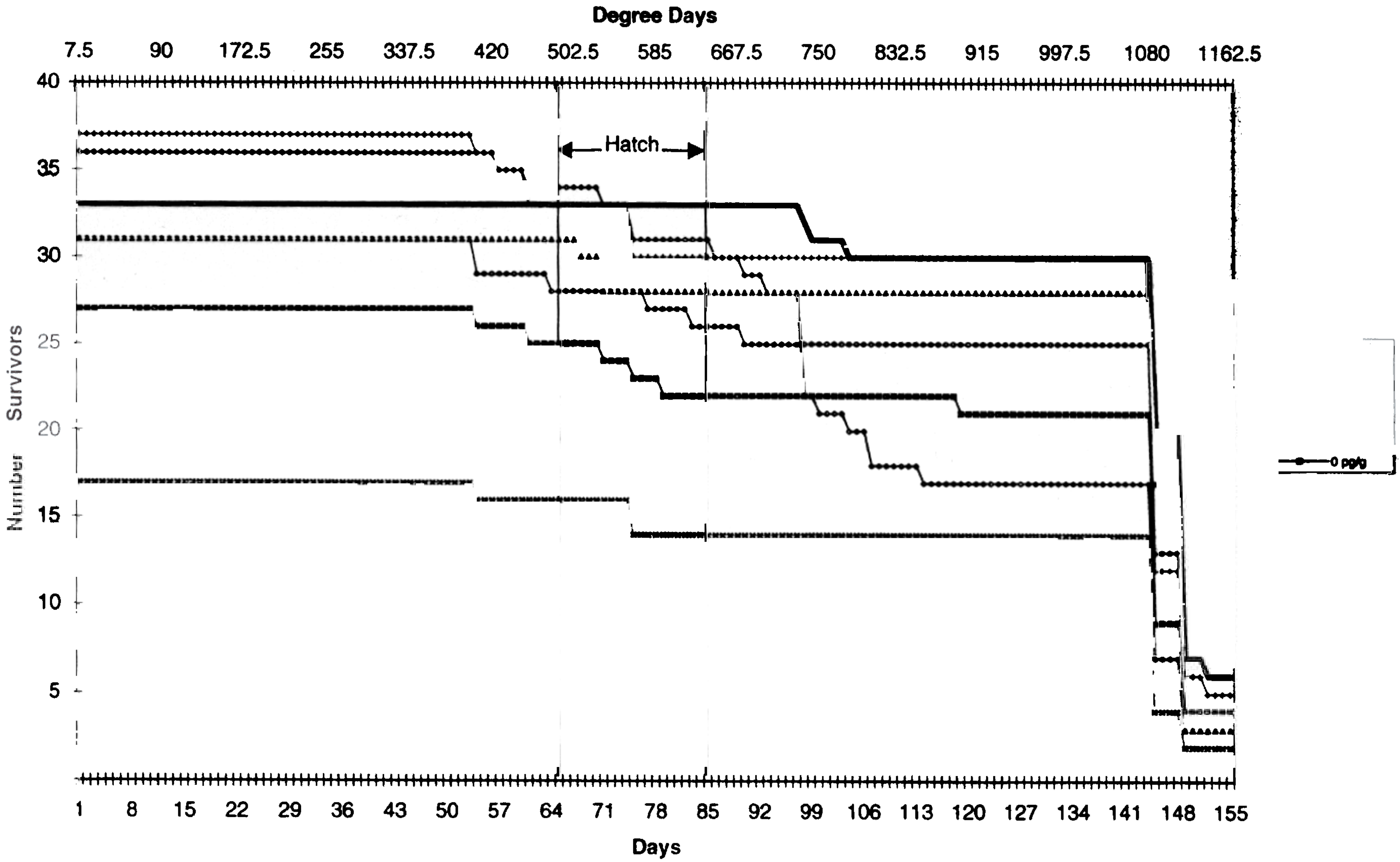
Survival of the TCDD injected fry was monitored and recorded (Fig. 5-8). The lake trout eggs which received no thiamine treatment at water hardening (Fig. 5-6) exhibited elevated mortality, prior to degree day 1080, at only the greatest dose of TCDD (122 pg/g egg). However, a large degree of swim-up mortality, presumably associated with low thiamine , was observed in these fry (Fig. 5). The large inflection in mortality around swim-up occurred at all doses of TCDD, only in the thiamine untreated lake trout fry (Fig. 5) and not in the lake trout which had been treated with 750 mg thiamine/L at water hardening (Fig. 7). Survival (%) prior to the swim-up mortality of the fry dosed with 122 pg TCDD/g egg was less in the fry without thiamine supplementation (Fig. 5), as compared to the mean survival in the fry with thiamine supplementation (Fig. 7). A monotonic dose-response was not observed in either of these studies with TCDD. The reason for this lack of a dose response is unknown. Only the greatest dose of TCDD resulted in an increase in the incidence of mortality over that of controls.

The other dose response study conducted in lake trout with TCDD was conducted in November and was unpaired for thiamine treatment. Eggs from the Wellsboro female T5-CBT-F12 were used for a dose response study (Fig. 9-10). Thiamine treatment during water hardening of the eggs resulted in a thaimine content of 4 nmol/g at 20 days post-fertilization (Table 5). As with the other set of TCDD dose response studies conducted here, only the greatest dose of TCDD resulted in elevated mortality in the lake trout fry (Fig. 9).

Gross pathologic lesions also did not occur in a dose-related fashion (data not shown).

Figure 5. Total Cumulative Survival (%) of Lake Trout Eggs and Fry From Wellsboro, With No Thiamine Treatment Exposed to 2,3,7,8-TCDD. Female/Egg Batch: 10/21 F-11-BT-T-0-1.





Degree Days
7.5
90
172.5
255
337.5
420
502.5
585
667.5
750
832.5
915
997.5
1080
1162.5
40
35
30
25
20
15
10
5
Number Survivors
Hatch
0 pg/g
1
8
15
22
29
36
43
50
57
64
71
78
85
92
99
106
113
120
127
134
141
148
155
Days

Figure 7. Total Cumulative Survival (%) of Lake Trout Eggs and Fry From Wellsboro, Treated With 750 mg/L Thiamine and Exposed to 2,3,7,8-TCDD. Female: 10/21 F-11-BT-T-1-1.

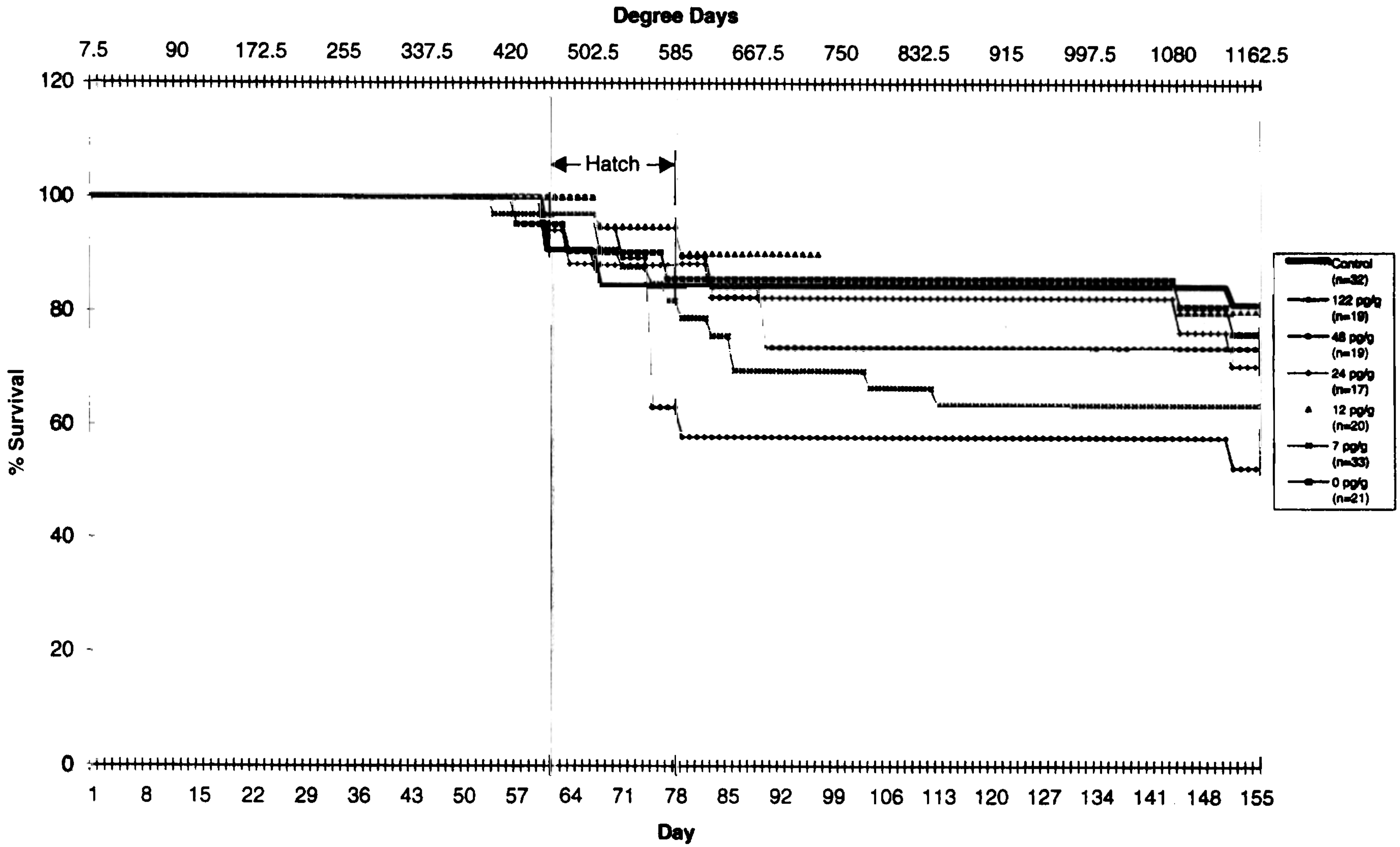


Figure 8. Total Cumulative Number of Survivors of Lake Trout Eggs and Fry From Wellsboro, Treated With 750 mg/L Thiamine and Exposed to 2,3,7,8-TCDD. Female/Egg Batch: 10/21 F-11-BT-T-1-1.

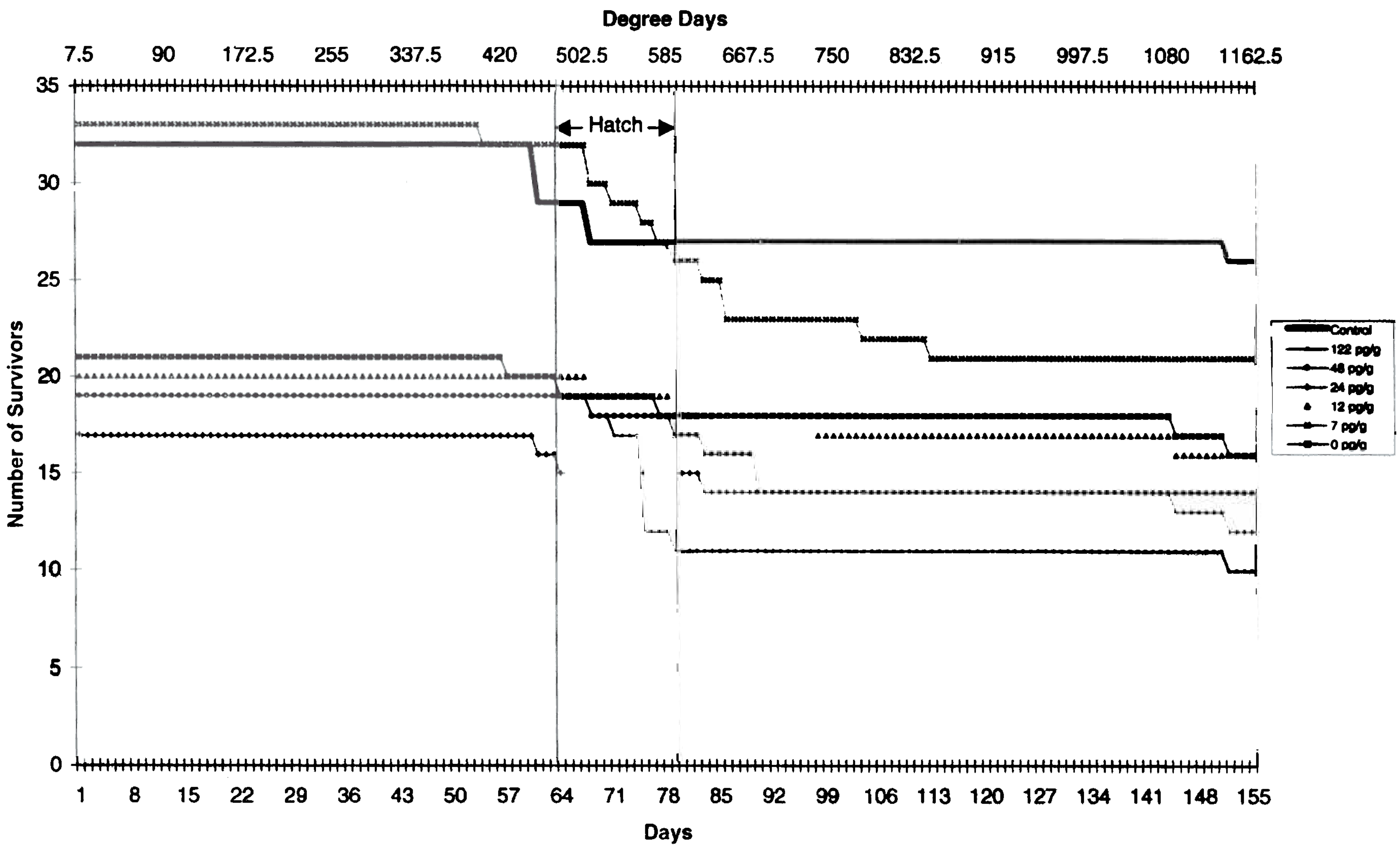


Figure 9. Total Cumulative Survival (%) of Lake Trout Eggs and Fry From Wellsboro, Treated With 750 mg/L Thiamine (nominal) and Exposed to 2,3,7,8-TCDD.
Female/Egg Batch: T5-CBT-F-12.

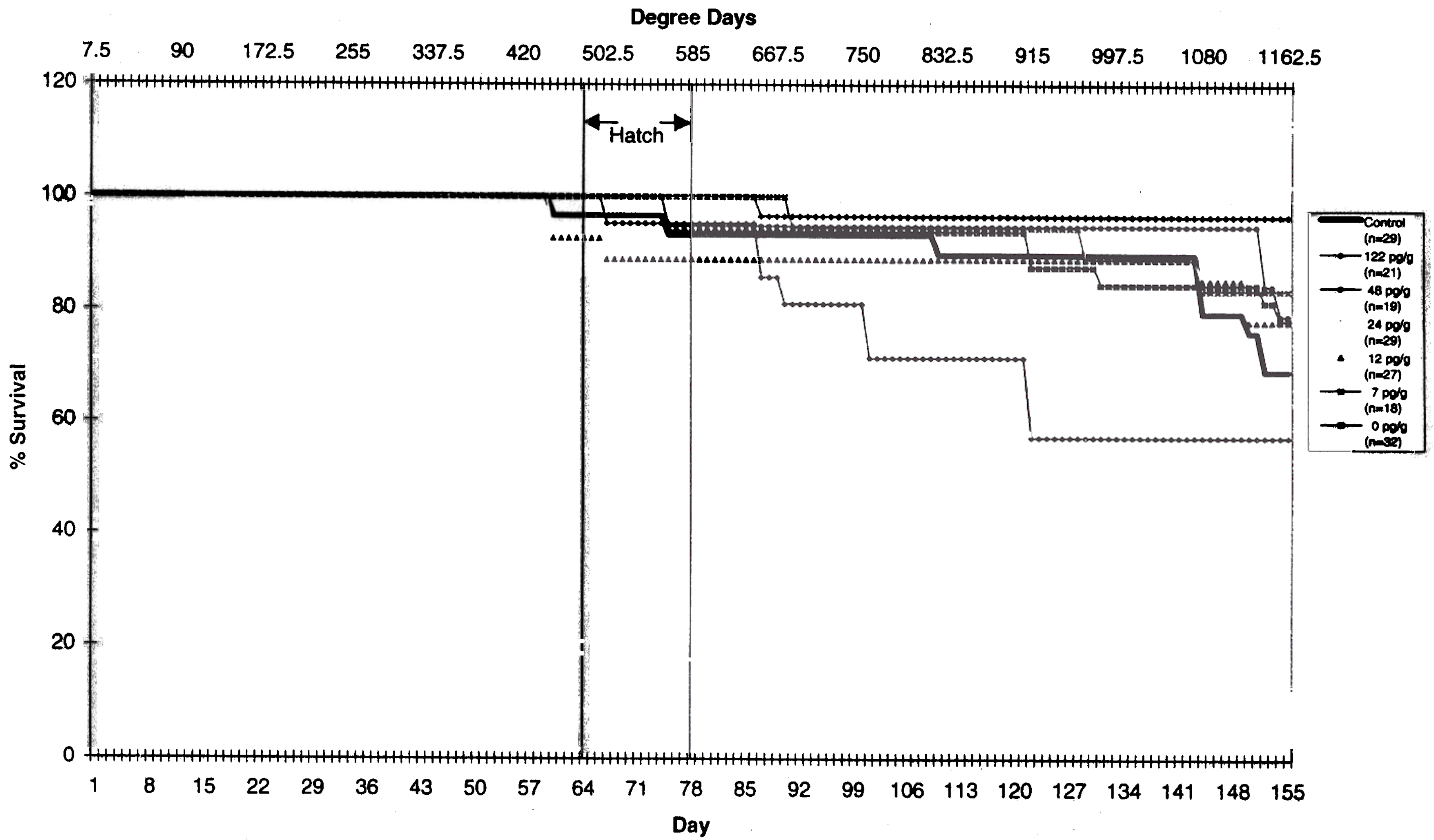
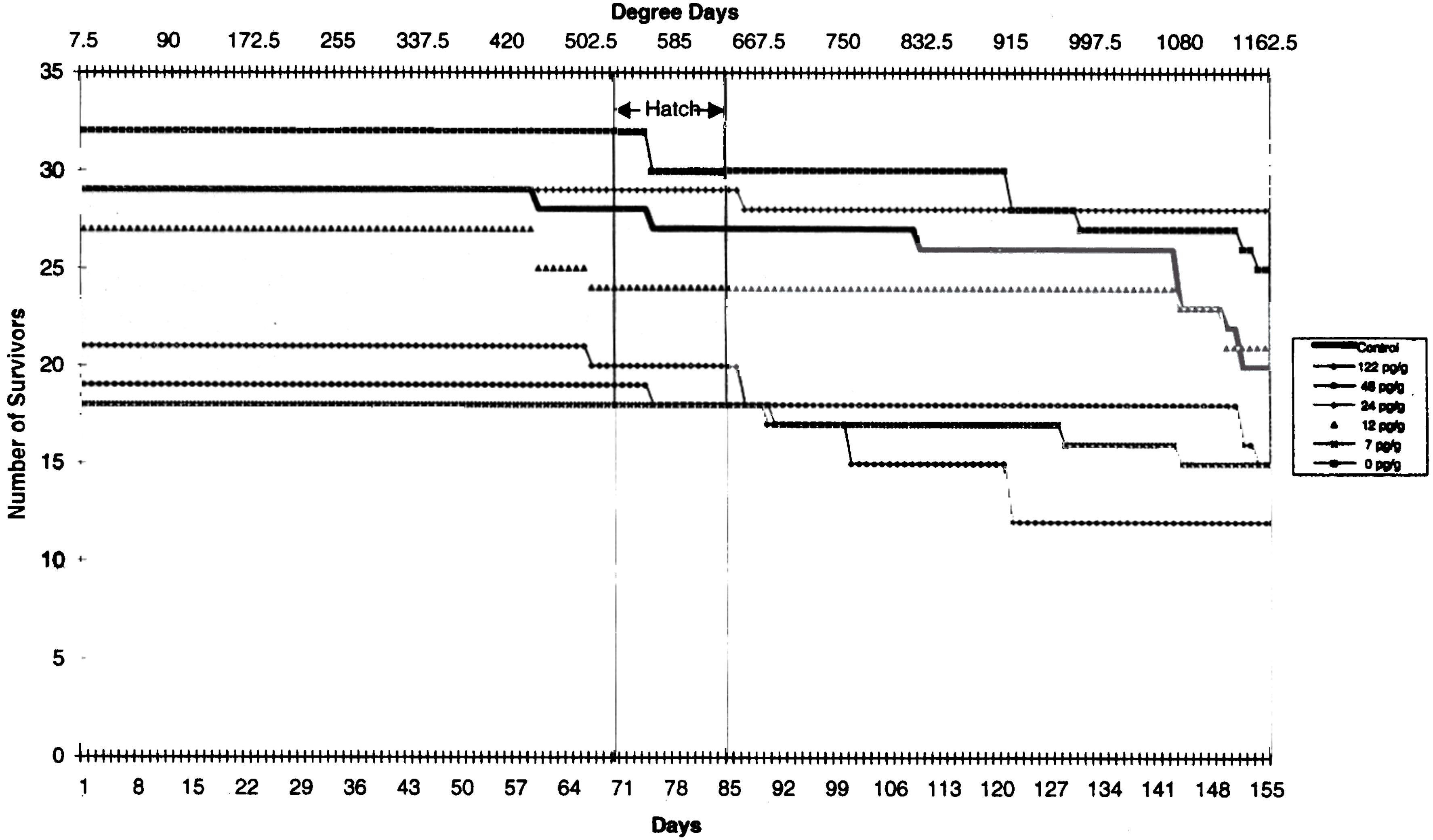


Figure 10. Total Cumulative Number of Survivors of Lake Trout Eggs and Fry From Wellsboro, Treated With 750 mg/L Thiamine (nominal) and Exposed to 2,3,7,8-TCDD.
Female/Egg Batch: T5-CBT-F-12.



Walleye Extract

The eggs from one Lake Michigan female (10/28-F10) and one Wellsboro female (T6-BT-F7) were used to conduct dose response studies with the Fox River/Green Bay Walleye Extract. The eggs from Lake Michigan lake trout (10/28-F10) had a pre-fertilization thiamine concentration of 2.1 nmol/g and the eggs from lake trout from the Wellsboro laboratory (T6-BT-F7) had a pre-fertilization thiamine level of 1.3 nmol/g. The eggs from the Lake Michigan female were treated at water hardening with 750 mg thiamine/L and the resultant concentration at 37 days post-fertilization was 10.3 nmol thiamine/g egg (Table 5). The eggs from the Wellsboro female used in the dosing studies with the walleye extract (T6-BT-F7) were split in two and treated with either 75 or 750 mg thiamine/L during water hardening. These thiamine treatments resulted in 1.7 and 7.5 nmol thiamine/g egg, respectively, after 28 days post-fertilization (Table 5).

The walleye extract was first tested for it toxic potency in the eggs from the Lake Michigan lake trout (10/28-F10). The doses of the extract ranged from 0.0045 to 45 eggEQ/egg or roughly one-half of one percent to 45 times the amount expected to be in lake trout eggs had they inhabited the same area as the walleye from the Fox River/Green Bay. The TEQ doses in these that result from the walleye extract ranged from 0.016 to 157 pg TEQs/g egg (Table 4). The mortality observed in the lake trout fry dosed with the walleye extract appeared to be elevated above controls in the top three doses (1.5, 15 and 157 pg TEQs/g egg added) (Fig.11-12). However, an analysis of variance and Dunnett's test suggested that only the greatest treatment (45 eggEQ or 157 pg TEQs/g egg) was significantly different from the controls. Cranio-facial malformations in surviving fry from the two highest doses (15 and 157 pg TEQs/g egg) were elevated over controls, but again, only the dose of 157 TEQs/g egg caused a statistically significant increase in the occurrence of this anomaly (data not presented). Therefore the threshold for effects on survival or gross pathologies was between 15 and 157 pg TEQs/g egg when the walleye extract was used in the egg of lake trout from Lake Michigan. Even though the statistical significance of the elevated cranio-facial anomalies was not suggested at the 15 pg TEQs/g egg, it is conceivable that this was due to the low number of samples in the treatment. The TEQs in the eggs of the Lake Michigan lake trout would be expected to be in the range of 5 pg TEQs/g, based on previous studies (Cook et al. 1997). Thus, the total amount of dioxin-like potency in these eggs would be in the range of 20 pg TEQs/g egg, which is thought to be at or above the threshold for dioxin-like effects in lake trout (Cook et al. 1997).

The other set of dose response studies conducted with the walleye extract were conducted with lake trout eggs from a Wellsboro female. The treatments at water hardening were 75 or 750 mg thiamine/L. The results were not unlike those with the lake trout egg from Lake Michigan in that only the greatest dose (157 pg TEQs/g egg) caused a significant amount of mortality above the controls (Fig. 13-16). The gross lesions (hemorrhage, yolk sac edema, and cranio-facial malformations) were also only significantly elevated at the greatest dose (data not presented).

Figure 11. Total Cumulative Survival (%) of Lake Trout Eggs and Fry From Lake Michigan, Treated With 750 mg/L Thiamine and Exposed to Walleye Extract From the Fox River/Green Bay. Female/Egg Batch: 10/28 F-10-F-T-1-1 .

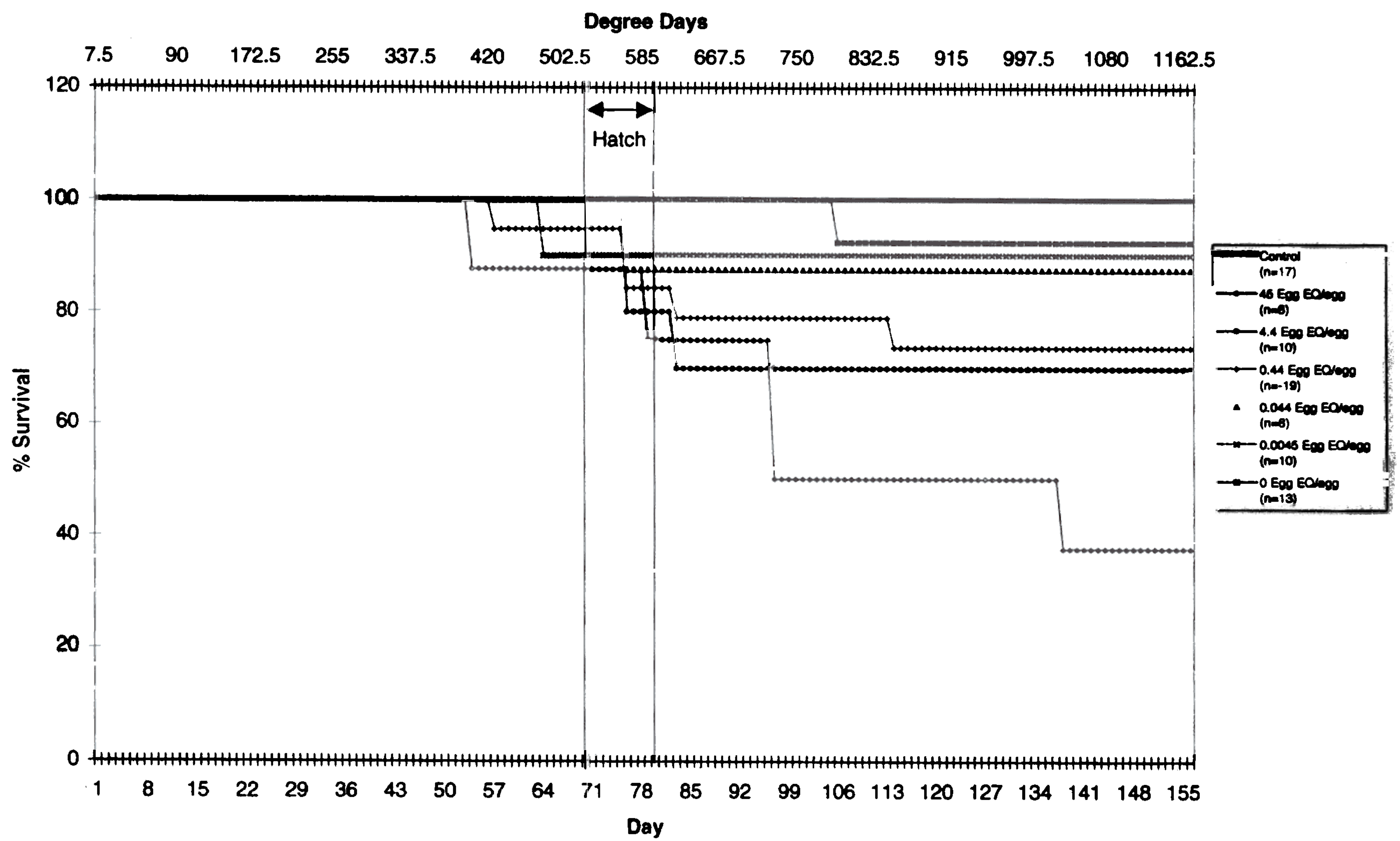


Figure 12. Total Cumulative Number of Survivors of Lake Trout Eggs and Fry From Lake Michigan, Treated With 750 mg/L Thiamine and Exposed to Walleye Extract From the Fox River/Green Bay. Female/Egg Batch: 10/28 F-10-F-T-1-1.

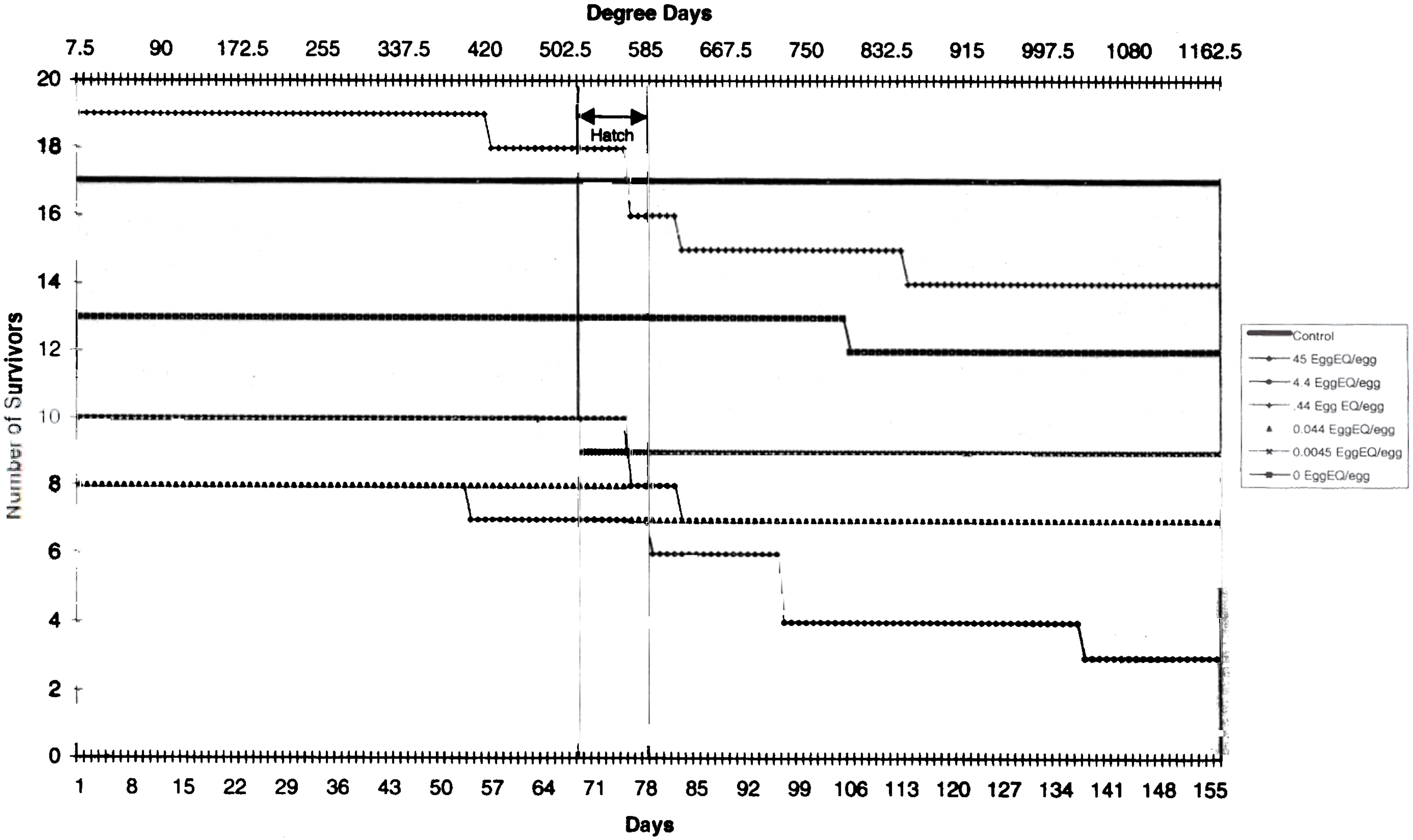


Figure 13. Total Cumulative Survival (%) of Lake Trout Eggs and Fry From Wellsboro, Treated With 75 mg/L Thiamine and Exposed to Walleye Extract From the Fox River/Green Bay.
Female/Egg Batch: T6-BT-F-7.

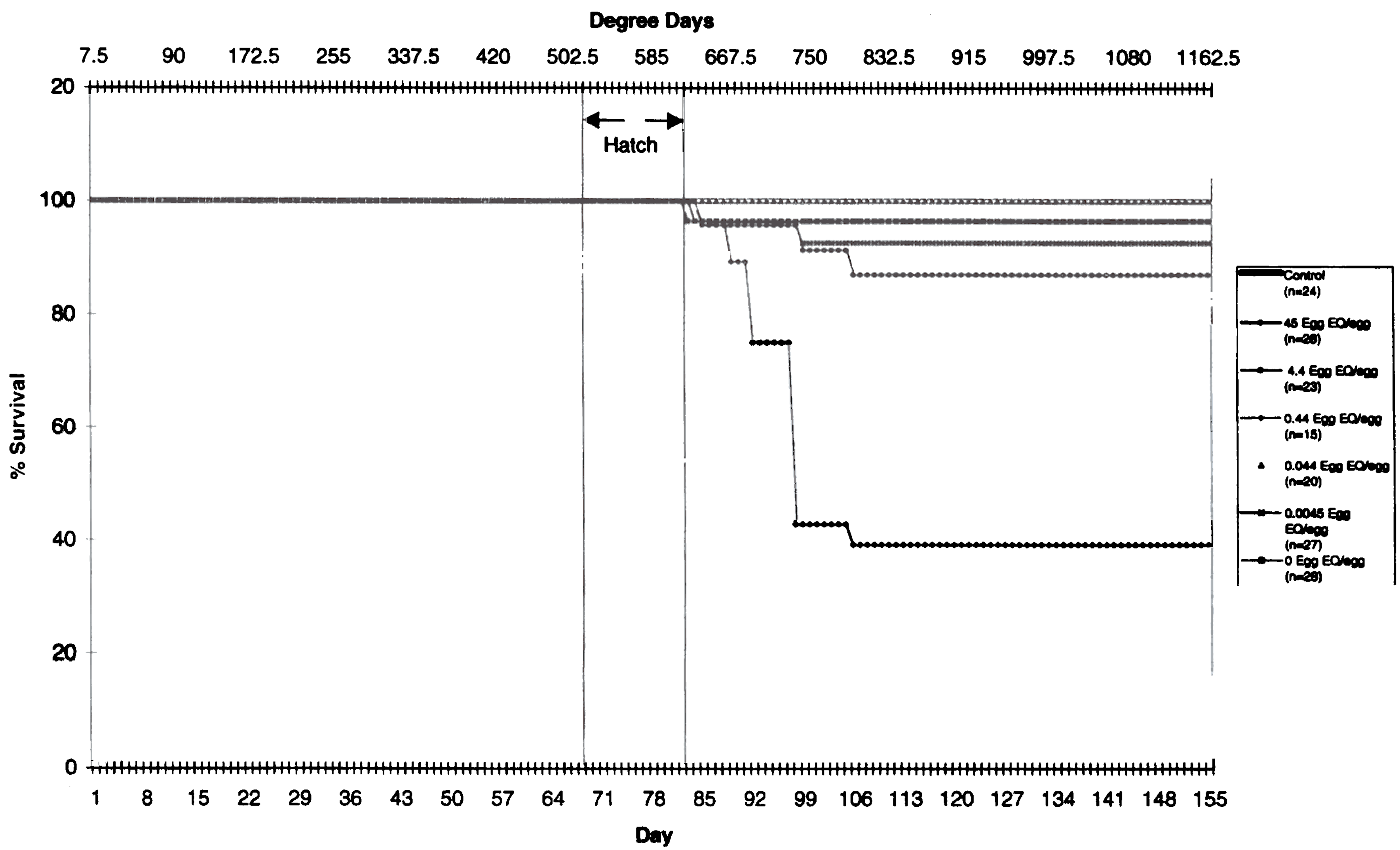


Figure 14. Total Cumulative Number of Survivors of Lake Trout Eggs and Fry From Wellsboro, Treated With 75mg/L Thiamine and Exposed to Walleye Extract From the Fox River/Green Bay. Female/Egg Batch: T6-BT-F-7.

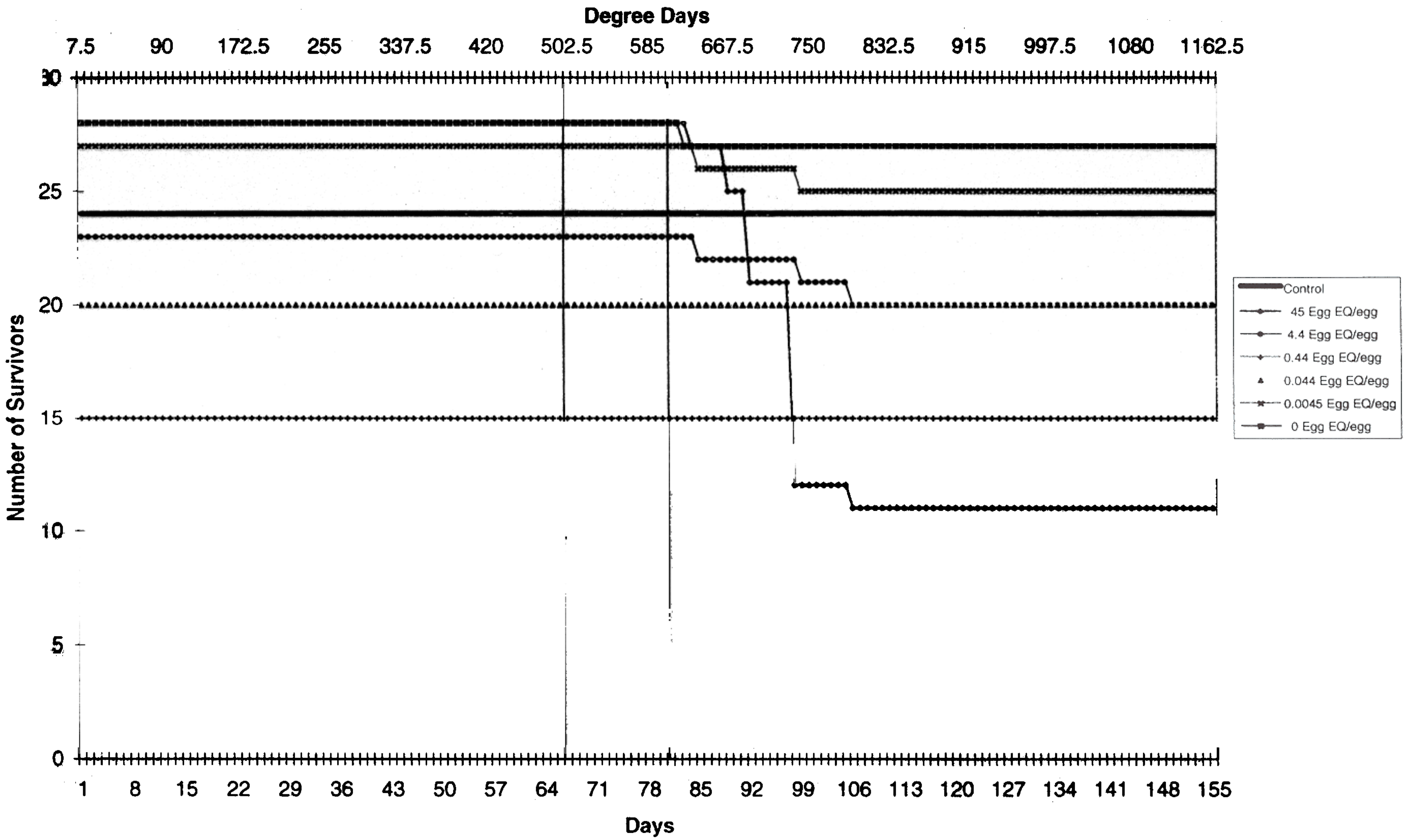


Figure 15. Total Cumulative Survival (%) of Lake Trout Eggs and Fry From Wellsboro, Treated With 750 mg/L Thiamine and Exposed to Walleye Extract From the Fox River/Green Bay. Female/Egg Batch: T6-BT-F-7.

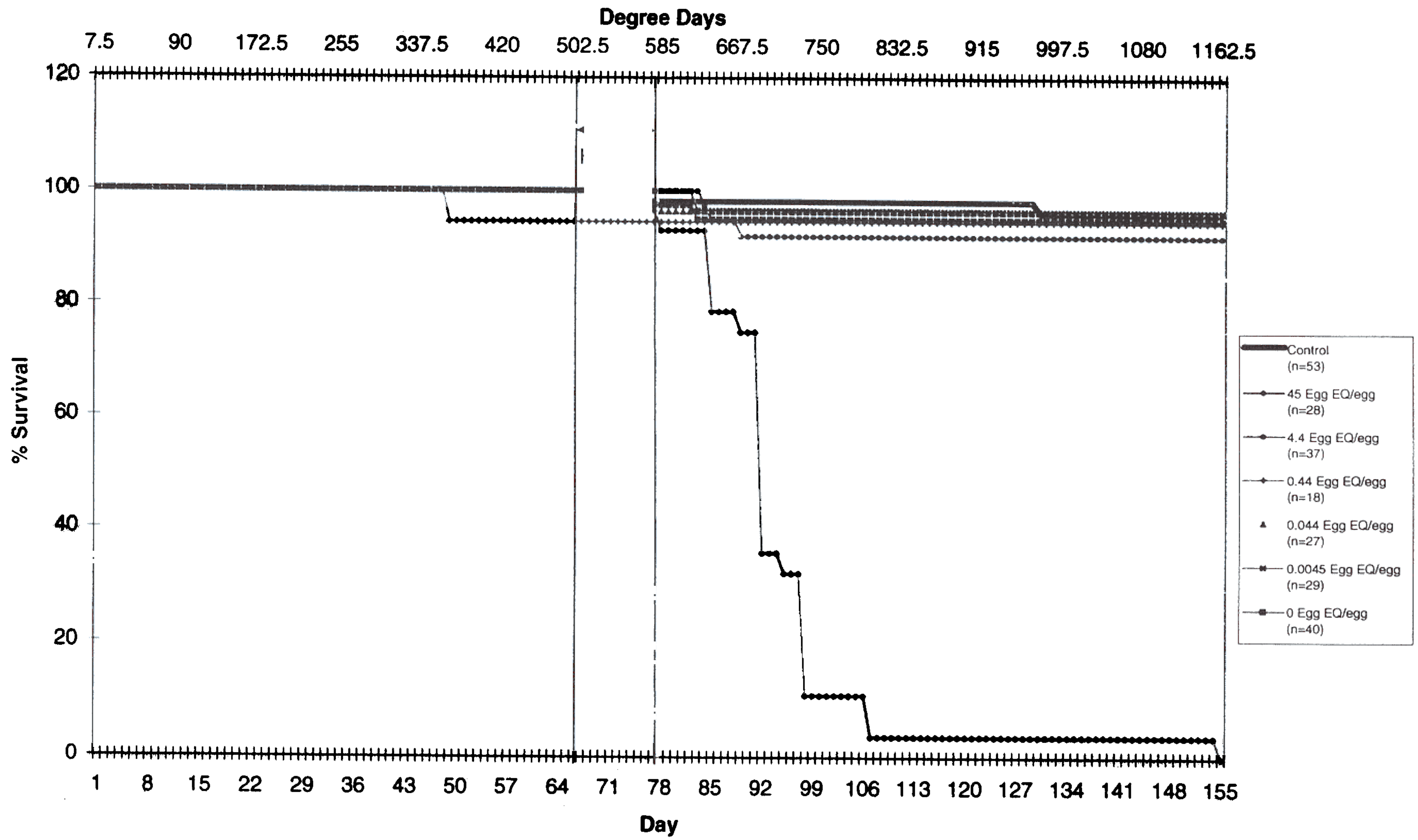
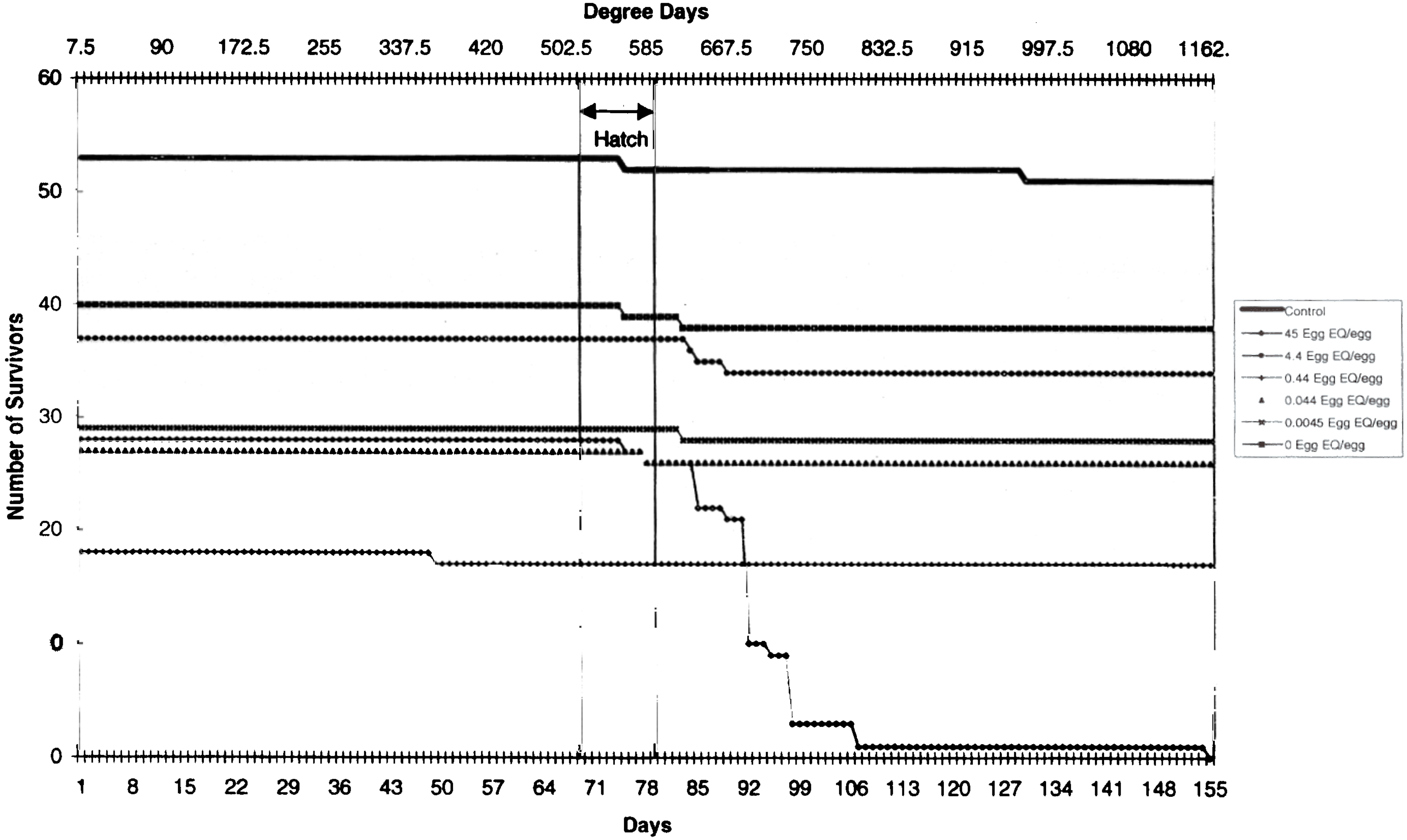


Figure 16. Total Cumulative Number of Survivors of Lake Trout Eggs and Fry From Wellsboro, Treated With 750mg/L Thiamine and Exposed to Walleye Extract From the Fox River/Green Bay. Female/Egg Batch: T6-BT-F-7.



Therefore, these studies confirm that a threshold for survival and gross pathologies in lake trout fry is between 15 and 157 pg TEQ/g egg (or 4.4 and 45 eggEQ of the walleye extract).

Conclusions and Recommendations

The sensitivity of lake trout toward TCDD, PCBs and other planar halogenated compounds was a key factor to instigate our studies to understand the potential for fish injury in Green Bay and the Fox River caused by PCB contamination. These studies confirmed the information in the scientific literature that establishes the lake trout as one of the most sensitive species toward the adverse affects of dioxin-like chemicals. However, these studies failed to support our original hypothesis that low thiamine status in lake trout eggs would further enhance the sensitivity of this species toward dioxin-like toxicity. The data did not support the contention that low thiamine content in lake trout embryos and fry might exacerbate the effects of PCBs or dioxins on fry survival. Various technical factors compromised the studies and a rigorous test of the hypothesis was not possible. However, certain conclusions may be taken from our work.

Dose related increases in fry mortality were observed with PCB 126 (3,3',4,4',5-pentachlorobiphenyl) and the median toxicity values obtained in our studies confirm the one study from the literature. The LD_{50} values of PCB 126 in lake trout fry were 20 and 27 ng/g egg. This compared well to the lone LD_{50} value in the literature for lake trout of 29 ng/g egg (Zabel et al. 1995).

The complex mixture of organic chemicals extracted from the Fox River walleye caused dioxin-like toxicity in early life stages of lake trout. The greatest dose (157 pg TEQs/g) caused deformities in all of the fry and nearly complete mortality. The next lower dose of the walleye extract (15 pg TEQs/g) caused increases in deformities and mortality in some groups, but the increases were not statistically significant. This dose of the walleye extract (15 pg TEQs/g) appears to be just below the threshold for dioxin-like toxicity for lake trout fry survival, which is again consistent with the literature. The result of the injection studies with the walleye extract are also consistent with an additive model of toxicity, and support the continued use of the TEF/TEQ approach for assessment of dioxin-like effects in developing lake trout.

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Last, the P.I. would like to acknowledge his appreciation of all the long hours and hard work that Dr. Alan Allert invested in this project. He was not given an easy task, yet he persisted to achieve the best product possible. Thanks.

5.0 Appendices

Raw Data on Mortality and Deformities